Connecting via Winsock to STN

Welcome to STN International! Enter x:x

LOGINID:SSSPTA1644PNH

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

* * * * * * * * * * Welcome to STN International * * * * * * * * * *

NEWS 1		Web Page URLs for STN Seminar Schedule - N. America
NEWS 2	Jan 25	BLAST(R) searching in REGISTRY available in STN on the Web
NEWS 3	Jan 29	FSTA has been reloaded and moves to weekly updates
NEWS 4	Feb 01	DKILIT now produced by FIZ Karlsruhe and has a new update frequency
NEWS 5	Feb 19	Access via Tymnet and SprintNet Eliminated Effective 3/31/02
NEWS 6	Mar 08	Gene Names now available in BIOSIS
NEWS 7	Mar 22	TOXLIT no longer available
NEWS 8	Mar 22	TRCTHERMO no longer available
NEWS 9	Mar 28	US Provisional Priorities searched with P in CA/CAplus and USPATFULL
NEWS 10	Mar 28	LIPINSKI/CALC added for property searching in REGISTRY
NEWS 11	Apr 02	PAPERCHEM no longer available on STN. Use PAPERCHEM2 instead.
NEWS 12	Apr 08	"Ask CAS" for self-help around the clock
NEWS 13	Apr 09	BEILSTEIN: Reload and Implementation of a New Subject Area
NEWS 14	Apr 09	ZDB will be removed from STN
NEWS 15	Apr 19	US Patent Applications available in IFICDB, IFIPAT, and IFIUDB
NEWS 16	Apr 22	Records from IP.com available in CAPLUS, HCAPLUS, and ZCAPLUS
NEWS 17	Apr 22	BIOSIS Gene Names now available in TOXCENTER
NEWS 18	Apr 22	Federal Research in Progress (FEDRIP) now available
NEWS 19	Jun 03	New e-mail delivery for search results now available
NEWS 20	Jun 10	MEDLINE Reload
NEWS 21	Jun 10	PCTFULL has been reloaded
NEWS 22	Jul 02	FOREGE no longer contains STANDARDS file segment
NEWS 23	Jul 19	NTIS to be reloaded July 28, 2002
NEWS 24	Jul 22	USAN to be reloaded July 28, 2002; saved answer sets no longer valid
NEWS 25	Jul 29	Enhanced polymer searching in REGISTRY
NEWS 26	Jul 30	NETFIRST to be removed from STN

NEWS EXPRESS	February 1 CURRENT WINDOWS VERSION IS V6.0d, CURRENT MACINTOSH VERSION IS V6.0a(ENG) AND V6.0Ja(JP), AND CURRENT DISCOVER FILE IS DATED 05 FEBRUARY 2002
NEWS HOURS	STN Operating Hours Plus Help Desk Availability
NEWS INTER	General Internet Information
NEWS LOGIN	Welcome Banner and News Items
NEWS PHONE	Direct Dial and Telecommunication Network Access to STN
NEWS WWW	CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that specific topic.

```
* * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *


FILE 'HOME' ENTERED AT 11:25:15 ON 05 AUG 2002

=> file medline embase biosis scisearch caplus
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILE 'MEDLINE' ENTERED AT 11:25:29 ON 05 AUG 2002

FILE 'EMBASE' ENTERED AT 11:25:29 ON 05 AUG 2002
COPYRIGHT (C) 2002 Elsevier Science B.V.  All rights reserved.

FILE 'BIOSIS' ENTERED AT 11:25:29 ON 05 AUG 2002
COPYRIGHT (C) 2002 BIOLOGICAL ABSTRACTS INC.(R)

FILE 'SCISEARCH' ENTERED AT 11:25:29 ON 05 AUG 2002
COPYRIGHT (C) 2002 Institute for Scientific Information (ISI) (R)

FILE 'CAPLUS' ENTERED AT 11:25:29 ON 05 AUG 2002
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2002 AMERICAN CHEMICAL SOCIETY (ACS)

=> s antibody
L1     2332137 ANTIBODY

=> s l1 and epidermal growth factor receptor
L2        8371 L1 AND EPIDERMAL GROWTH FACTOR RECEPTOR

=> s l2 and EGFR
L3        3081 L2 AND EGFR

=> s l3 and threonine phosphorylation
L4           0 L3 AND THREONINE PHOSPHORYLATION

=> s l3 and phosphorylation
L5         553 L3 AND PHOSPHORYLATION

=> s l5 and serine
L6          18 L5 AND SERINE

=> dup remove l6
PROCESSING COMPLETED FOR L6
L7          10 DUP REMOVE L6 (8 DUPLICATES REMOVED)

=> d l7 1-10 cbib abs

L7   ANSWER 1 OF 10     MEDLINE
2002285242  Document Number: 22021454.    PubMed ID: 11992543.   Effects of
     pharmacologic antagonists of epidermal growth
     factor receptor, PI3K and MEK signal kinases on
     NF-kappaB and AP-1 activation and IL-8 and VEGF expression in human head
     and neck squamous cell carcinoma lines. Bancroft Caren C; Chen Zhong; Yeh
     Jason; Sunwoo John B; Yeh Ning T; Jackson Sadhana; Jackson Chad; Van Waes
     Carter. (Tumor Biology Section, Head and Neck Surgery Branch, The National
     Institute on Deafness and Other Communication Disorders, The National
     Institutes of Health, Bethesda, MD 20892, USA. ) INTERNATIONAL JOURNAL OF
     CANCER, (2002 Jun 1) 99 (4) 538-48.  Journal code: 0042124. ISSN:
```

0020-7136. Pub. country: United States. Language: English.

AB We previously reported that expression of angiogenesis factors interleukin-8 (IL-8) and vascular endothelial growth factor (VEGF) is promoted by coactivation of transcription factors nuclear factor-kappaB (NF-kappaB) and activator protein-1 (AP-1) by interleukin-1alpha in human head and neck squamous cell carcinomas (HNSCC). However, expression of IL-1 receptor antagonist incompletely blocked reporter gene activity and cytokine expression, suggesting that other upstream signals may contribute to activation. Overexpression and autocrine activation of **epidermal growth factor receptor** (**EGFR**) is detected in 90% of HNSCC, and **EGFR** inhibitors have been reported to inhibit IL-8 and VEGF expression, but the intermediary signal pathways and transcription factors by which **EGFR** modulates proangiogenic factors is unknown. **EGFR** can activate the phosphotidylinositol-3 kinase (PI3K) and mitogen-activated/extracellular signal-regulated kinase (MEK) pathways, which can potentially modulate activation of NF-kappaB and AP-1, respectively. In our study, we examined the effect of EGF and antagonists of **EGFR**, PI3K and MEK on NF-kappaB and AP-1 activation and IL-8 and VEGF expression in HNSCC cell lines UM-SCC-9 and 11B in which **EGFR** is overexpressed and activated. Recombinant EGF induced **EGFR phosphorylation**, activation of NF-kappaB and AP-1 reporter genes and IL-8 and VEGF expression, indicating that **EGFR** can mediate coactivation of both transcription factors and cytokine genes in HNSCC. **EGFR** antagonist PD153035 and anti-**EGFR** **antibody** C225 completely inhibited EGF-induced reporter activity and cytokine expression, but only partially inhibited constitutive activity. MEK inhibitor U0126 preferentially blocked AP-1 activity and expression of both IL-8 and VEGF, while PI3K inhibitor LY-294002 or a dominant negative inhibitor-kappaB preferentially blocked NF-kappaB activation and expression of IL-8 but not VEGF. **EGFR**, PI3K and MEK antagonists inhibited growth of HNSCC. We conclude that antagonists of **EGFR**, PI3K and MEK signal pathways have inhibitory activity against **EGFR**-induced NF-kappaB and AP-1 activation, IL-8 and VEGF expression and growth by HNSCC. Published 2002 Wiley-Liss, Inc.

L7 ANSWER 2 OF 10 MEDLINE

2002102880 Document Number: 21821829. PubMed ID: 11832328.

Vasopressin-mediated mitogenic signaling in intestinal epithelial cells. Chiu Terence; Wu Steven S; Santiskulvong Chintda; Tangkijvanich Pisit; Yee Hal F Jr; Rozengurt Enrique. (Department of Medicine, School of Medicine, University of California-Los Angeles, 900 Veteran Ave., Los Angeles, CA 90095, USA.) AMERICAN JOURNAL OF PHYSIOLOGY. CELL PHYSIOLOGY, (2002 Mar) 282 (3) C434-50. Journal code: 100901225. ISSN: 0363-6143. Pub. country: United States. Language: English.

AB The role of G protein-coupled receptors and their ligands in intestinal epithelial cell signaling and proliferation is poorly understood. Here, we demonstrate that arginine vasopressin (AVP) induces multiple intracellular signal transduction pathways in rat intestinal epithelial IEC-18 cells via a V(1A) receptor. Addition of AVP to these cells induces a rapid and transient increase in cytosolic Ca(2+) concentration and promotes protein kinase D (PKD) activation through a protein kinase C (PKC)-dependent pathway, as revealed by in vitro kinase assays and immunoblotting with an **antibody** that recognizes autophosphorylated PKD at Ser(916). AVP also stimulates the tyrosine **phosphorylation** of the nonreceptor tyrosine kinase proline-rich tyrosine kinase 2 (Pyk2) and promotes Src family kinase **phosphorylation** at Tyr(418), indicative of Src activation. AVP induces extracellular signal-related kinase (ERK)-1 (p44(mapk)) and ERK-2 (p42(mapk)) activation, a response prevented by treatment with mitogen-activated protein kinase kinase (MEK) inhibitors (PD-98059 and U-0126), specific PKC inhibitors (GF-I and Ro-31-8220), depletion of Ca(2+) (EGTA and thapsigargin), selective **epidermal growth factor receptor** (**EGFR**)

tyrosine kinase inhibitors (tyrphostin AG-1478, compound 56), or the selective Src family kinase inhibitor PP-2. Furthermore, AVP acts as a potent growth factor for IEC-18 cells, inducing DNA synthesis and cell proliferation through ERK-, Ca(2+)-, PKC-, **EGFR** tyrosine kinase-, and Src-dependent pathways.

L7 ANSWER 3 OF 10 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2002:271598 Document No.: PREV200200271598. Epidermal growth factor and thrombin induced proliferation of immortalized human keratinocytes is coupled to the synthesis of Egr-1, a zinc finger transcriptional regulator. Kaufmann, Katrin; Thiel, Gerald (1). (1) Department of Medical Biochemistry and Molecular Biology, University of Saarland Medical Center, Building 44, D-66421, Homburg: bcgthi@uniklinik-saarland.de Germany. Journal of Cellular Biochemistry, (2002) Vol. 85, No. 2, pp. 381-391. http://www.interscience.wiley.com/jpages/0730-2312/. print. ISSN: 0730-2312. Language: English.

AB The epidermal growth factor (EGF) receptor is highly expressed in HaCaT keratinocytes as shown by Western blotting. Stimulation of HaCaT cells with EGF, and also with the **serine** protease thrombin, induced DNA synthesis, measured by incorporation of 5-bromo-2'-deoxyuridine into the DNA of proliferating cells. Using **antibodies** directed against the active form of the EGF receptor, we show that in HaCaT cells EGF and thrombin triggered a rapid activation of the EGF receptor, followed by the **phosphorylation** and activation of the extracellular signal-regulated protein kinase (ERK). Moreover, EGF and thrombin induced a transient synthesis of the zinc finger transcriptional regulator Egr-1. Proliferation, activation of ERK, and biosynthesis of Egr-1 was completely inhibited in EGF or thrombin-treated HaCaT cells by the MAP kinase kinase inhibitor PD98059 and by AG1487, an EGF receptor-specific tyrosine kinase inhibitor. These data indicate that **phosphorylation** and activation of both the EGF receptor and ERK are essential for mitogenic signaling via EGF and thrombin. The synthesis of Egr-1 in HaCaT cells as a result of EGF or thrombin stimulation suggests that Egr-1 may be an important "late" part of the EGF and thrombin-initiated signaling cascades. We postulate that Egr-1 may function as a "third messenger" in keratinocytes connecting mitogenic stimulation with changes in gene transcription.

L7 ANSWER 4 OF 10 MEDLINE DUPLICATE 1
2001526609 Document Number: 21228271. PubMed ID: 11330837. Association of ErbB2 Ser1113 **phosphorylation** with **epidermal growth factor receptor** co-expression and poor prognosis in human breast cancer. Ouyang X; Gulliford T; Zhang H; Smith G; Huang G; Epstein R J. (Department of Metabolic Medicine, Imperial College School of Medicine, London, UK.) MOLECULAR AND CELLULAR BIOCHEMISTRY, (2001 Feb) 218 (1-2) 47-54. Journal code: 0364456. ISSN: 0300-8177. Pub. country: Netherlands. Language: English.

AB The carboxyterminal domain of the **epidermal growth factor receptor** (**EGFR**)--a putative binding site for the ubiquitin ligase Cbl--is the site of **serine phosphorylation** events which are essential for ligand-dependent **EGFR** desensitization and degradation. Using a monoclonal **antibody** (aPS1113) which selectively recognizes the homologous phosphorylated domain in the ErbB2 oncoprotein, we show here that wild-type ErbB2 becomes Ser1113-phosphorylated following treatment of 3T3 cells with growth factors or tyrosine phosphatase inhibitors. In **EGFR**-overexpressing A431 cells, ligand-inducible aPS1113 immunoreactivity declines more rapidly than other detectable **phosphorylation** events and is followed by **EGFR** downregulation. Analysis of 65 ErbB2-expressing primary breast cancers reveals a highly significant relationship between Ser1113 **phosphorylation** and **EGFR** overexpression ($p < 0.0001$) as well as an association with poor prognosis ($p = 0.005$). We submit that

ErbB2 Ser1113 **phosphorylation** status represents a novel and informative biomarker of cancer cell biology and tumor behavior.

L7 ANSWER 5 OF 10 MEDLINE

2000404731 Document Number: 20357377. PubMed ID: 10801894. Peroxynitrite targets the **epidermal growth factor receptor**, Raf-1, and MEK independently to activate MAPK. Zhang P; Wang Y Z; Kagan E; Bonner J C. (Laboratory of Pulmonary Pathobiology, NIEHS, National Institutes of Health, Research Triangle Park, North Carolina 27709, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Jul 21) 275 (29) 22479-86. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Activation of ERK-1 and -2 by H(2)O(2) in a variety of cell types requires **epidermal growth factor receptor** (**EGFR**) **phosphorylation**. In this study, we investigated the activation of ERK by ONOO(-) in cultured rat lung myofibroblasts. Western blot analysis using anti-phospho-ERK **antibodies** along with an ERK kinase assay using the phosphorylated heat- and acid-stable protein (PHAS-1) substrate demonstrated that ERK activation peaked within 15 min after ONOO(-) treatment and was maximally activated with 100 micrometer ONOO(-). Activation of ERK by ONOO(-) and H(2)O(2) was blocked by the antioxidant N-acetyl-l-cysteine. Catalase blocked ERK activation by H(2)O(2), but not by ONOO(-), demonstrating that the effect of ONOO(-) was not due to the generation of H(2)O(2). Both H(2)O(2) and ONOO(-) induced **phosphorylation** of **EGFR** in Western blot experiments using an anti-phospho-**EGFR antibody**. However, the **EGFR** tyrosine kinase inhibitor AG1478 abolished ERK activation by H(2)O(2), but not by ONOO(-). Both H(2)O(2) and ONOO(-) activated Raf-1. However, the Raf inhibitor forskolin blocked ERK activation by H(2)O(2), but not by ONOO(-). The MEK inhibitor PD98059 inhibited ERK activation by both H(2)O(2) and ONOO(-). Moreover, ONOO(-) or H(2)O(2) caused a cytotoxic response of myofibroblasts that was prevented by preincubation with PD98059. In a cell-free kinase assay, ONOO(-) (but not H(2)O(2)) induced autophosphorylation and nitration of a glutathione S-transferase-MEK-1 fusion protein. Collectively, these data indicate that ONOO(-) activates **EGFR** and Raf-1, but these signaling intermediates are not required for ONOO(-)-induced ERK activation. However, MEK-1 activation is required for ONOO(-)-induced ERK activation in myofibroblasts. In contrast, H(2)O(2)-induced ERK activation is dependent on **EGFR** activation, which then leads to downstream Raf-1 and MEK-1 activation.

L7 ANSWER 6 OF 10 MEDLINE

1999232131 Document Number: 99232131. PubMed ID: 10216485. Investigation of the Mek-MAP kinase-Rsk pathway in human breast cancer. Salh B; Marotta A; Matthewson C; Ahluwalia M; Flint J; Owen D; Pelech S. (Department of Medicine, University of British Columbia, Vancouver, Canada.) ANTICANCER RESEARCH, (1999 Jan-Feb) 19 (1B) 731-40. Journal code: 8102988. ISSN: 0250-7005. Pub. country: Greece. Language: English.

AB BACKGROUND: Mitogenic signaling through the principal growth factor receptor tyrosine kinase (RTK) pathway, i.e. RTK-->Ras-->Raf-->Mek-->MAPK has been implicated in the pathogenesis of human cancer. However, biochemical characterization of this has not been adequately assessed in human cancers. MATERIALS AND METHODS: Using extracts from 23 human breast cancers and control tissue from the same resected specimens, the protein levels, phosphotransferase activities and subcellular locations of the mitogen-activated protein (MAP) kinase isoforms p42 Erk2 and p44 Erk1 were examined, together with their phosphotransferase activities towards myelin basic protein (MBP) and a peptide substrate patterned after the Thr-669 site in the **epidermal growth factor receptor** (**EGFR** T669) that is phosphorylated by MAP kinase. RESULTS: Overexpression of both Erk2 and Erk1 isoforms was evident using specific **antibodies**. A universal activation of MBP and

EGFR T669 peptide phosphotransferase activities was also found (up to 3-fold). MonoQ fractionation resolved the bulk of the **EGFR** T669 peptide **phosphorylation** from elution of the MAP kinase protein. Erk1 and Erk2 activities determined by specific immunoprecipitation were increased by up to only 2.5-fold in only 50% of tumors overall. Immunohistochemical studies, using a monoclonal **antibody** specific for Erk2 demonstrated that the cellular distribution of this MAP kinase was similar in both control and tumor tissues, and Erk2 was largely confined to normal and malignant acini, whilst the intensity of staining was actually reduced in the tumor tissue. Mek1 and especially Mek2 protein expression, as well as MAP kinase kinase activity as determined by **phosphorylation** of kinase-inactive Erk [GST-K71A] were increased in cancer samples. CONCLUSIONS: a) This confirms that MAP kinase activity is increased in human breast cancer. However, the frequency and magnitude of this change is dependent upon the chosen methodology (i.e. crude lysate assays versus specific immunoprecipitation). b) A MAP-kinase-independent source of increased **EGFR** T669 phosphotransferase activity in tumor extracts has been demonstrated for the first time in human breast cancer. c) By immunohistochemistry, Erk2 protein was actually found to exhibit lower intensity in tumor samples; the increased expression was most likely due to its increased distribution. d) Increased Mek protein expression and activation have been demonstrated for the first time in human breast tumors.

L7 ANSWER 7 OF 10 SCISEARCH COPYRIGHT 2002 ISI (R)
96:673872 The Genuine Article (R) Number: VG672. MUTATIONAL ANALYSIS OF THE NUCLEOTIDE-BINDING SITE OF THE **EPIDERMAL GROWTH-FACTOR RECEPTOR** AND V-SRC PROTEIN-TYROSINE KINASES.
CHAN C L; GILL G N (Reprint). UNIV CALIF SAN DIEGO, DEPT MED, 9500 GILMAN DR, LA JOLLA, CA, 92093 (Reprint); UNIV CALIF SAN DIEGO, DEPT MED, LA JOLLA, CA, 92093. JOURNAL OF BIOLOGICAL CHEMISTRY (13 SEP 1996) Vol. 271, No. 37, pp. 22619-22623. ISSN: 0021-9258. Pub. country: USA. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Tyrosine kinases differ from **serine**/threonine kinases in sequences located at the active site where ATP and substrate bind. In the structure of cyclic AMP-dependent protein kinase, the catalytic loop contains the sequence Lys-Pro-Glu where the Lys residue contacts the gamma-phosphate of ATP and the Glu residue contacts a basic residue located in the peptide substrate. In tyrosine kinases, the analogous sequence is Ala-Ala-Arg in the receptor tyrosine kinase subfamily and Arg-Ala-Ala in the Src tyrosine kinase subfamily. To deduce the role of these residues in tyrosine kinase function, site-directed mutations were prepared in the **epidermal growth factor receptor** (**EGFR**) and in v-Src and effects on ATP binding and kinase activity were determined. Changing Arg to either Lys or Ala dramatically reduced activity of both tyrosine kinases and this correlated with loss of ATP binding. Changing the orientation of this sequence impaired activity of **EGFR** to a greater extent than that of v-Src but did not change substrate specificity of the two enzymes. These results support the hypothesis that Arg functions to coordinate the gamma-phosphate of ATP. Analysis of sequence inversions in the catalytic loop indicate that the active site of v-Src exhibits greater flexibility than that of **EGFR**.

L7 ANSWER 8 OF 10 MEDLINE DUPLICATE 2
96183364 Document Number: 96183364. PubMed ID: 8610433. The protein kinase activity of the large subunit of herpes simplex virus type 2 ribonucleotide reductase (ICP10) fused to the extracellular domain of the **epidermal growth factor receptor** is ligand-inducible. Smith C C; Luo J H; Aurelian L. (Department of Pharmacology, University of Maryland School of Medicine, Baltimore 21201,

USA.) VIROLOGY, (1996 Mar 15) 217 (2) 425-34. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The gene coding for the large subunit of herpes simplex virus type 2 ribonucleotide reductase (RR) (ICP10) has a unique 5' terminal domain the product of which has a **serine**/threonine (Ser/Thr) protein kinase (PK) catalytic domain preceded by a transmembrane (TM) segment. Because ICP10 localizes on the cell surface and is internalized by the endocytic pathway like an activated growth factor receptor (Hunter et al., 1995, Virology 210, 345-360), we asked whether it is ligand-inducible in order to examine whether it has intrinsic transphosphorylating activity. We constructed a chimeric expression vector that contains the extracellular and TM domains of the **epidermal growth factor receptor** (**EGFR**) joined to the intracellular PK and RR domains of ICP10 (pCH5) and established constitutively expressing cell lines in NIH3T3 2.2 cells that do not express **EGFR**. The chimeric protein, designated p210 CH5, localized to the surface of these cells as determined by immunofluorescent staining with MAb **EGFR**, and it bound 125I-EGF.p210 CH5 coprecipitated with protein species p170, p120, p88, p60, p44, p34, and p25. EGF treatment activated the PK activity of p210 CH5, resulting in its autophosphorylation and the **phosphorylation** of the p120, p88, and p34 species. Immunoprecipitation/immunoblotting with anti-ras-GAP **antibody** and phosphoamino acid analysis indicated that p120 is ras-GAP and it is phosphorylated on Ser/Thr residues. The identities of the phosphorylated p88 and p34 are still unknown. The data indicate that when fused to a ligand-regulated extracellular domain (**EGFR**), the ICP10 PK auto- and transphosphorylating activities are ligand-inducible. These findings support the interpretation that the ICP10 PK activity is intrinsic and indicate that ras-GAP is one of its **phosphorylation** substrates.

L7 ANSWER 9 OF 10 SCISEARCH COPYRIGHT 2002 ISI (R)

92:371621 The Genuine Article (R) Number: HY947. LIGAND-INDUCED ACTIVATION OF **EPIDERMAL GROWTH-FACTOR RECEPTOR** IN INTACT RAT MAMMARY ADENOCARCINOMA CELLS WITHOUT DETECTABLE RECEPTOR **PHOSPHORYLATION**. LICHTNER R B (Reprint); WIEDEMUTH M; KITTMANN A; ULLRICH A; SCHIRRMACHER V; KHAZAIE K. GERMAN CANC RES CTR, DEPT IMMUNOL & GENET, W-6900 HEIDELBERG 1, GERMANY; MAX PLANCK INST BIOCHEM, W-8033 MARTINSRIED, GERMANY. JOURNAL OF BIOLOGICAL CHEMISTRY (15 JUN 1992) Vol. 267, No. 17, pp. 11872-11880. ISSN: 0021-9258. Pub. country: GERMANY. Language: ENGLISH.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Expression and function of **epidermal growth factor receptor** (**EGFR**) was investigated in a metastatic cell clone (MTLn3) derived from the 13762NF rat mammary adenocarcinoma. No receptor **phosphorylation** could be identified in intact cells or in membrane preparations, while EGF-dependent **phosphorylation** of substrates occurred in intact cells. Indications for active suppression of receptor **phosphorylation** came from the fact that **EGFRs** bound in immunocomplexes or associated with the cytoskeleton of detergent treated cells were able to undergo basal and EGF-induced **phosphorylation** in vitro. Cross-linking experiments with I-125-EGF, as well as [S-35]methionine labeling followed by immunoprecipitation with receptor specific **antibodies** readily detected in MTLn3 cells the expected 170-kDa **EGFR** protein. In addition, two proteins with molecular masses of 420-480 and 95 kDa specifically bound I-125-EGF on intact MTLn3 and sparse cultures of A431 cells. **Phosphorylation** of the 420-480 kDa molecule could be identified in immunocomplexes of **EGFRs** isolated from MTLn3 and sparse A431 cells, but the 95-kDa receptor molecule was never phosphorylated. While the presence of alternative forms of **EGFR** in the highly metastatic cell clone MTLn3 was unexpected, our observations of inefficient receptor autophosphorylation are in agreement with other recent reports and suggest that in MTLn3 cells

EGFR-mediated signal transduction can be an event independent from receptor autophosphorylation.

L7 ANSWER 10 OF 10 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
92287258 EMBASE Document No.: 1992287258. Epidermal growth factor stimulates tyrosine **phosphorylation** in the neonatal mouse: Association of a M(r) 55,000 substrate with the receptor. Donaldson R.W.; Cohen S.. Department of Biochemistry, Vanderbilt Univ. School of Medicine,Nashville, TN 37232, United States. Proceedings of the National Academy of Sciences of the United States of America 89/18 (8477-8481) 1992. ISSN: 0027-8424. CODEN: PNASA6. Pub. Country: United States. Language: English. Summary Language: English.
AB Administration of epidermal growth factor (EGF) to neonatal mice resulted in rapid tyrosine **phosphorylation** of a number of specific substrates in liver, kidney, lung, bladder, skin, and brain as detected by Western blot analysis of tissue homogenates with anti-phosphotyrosine **antibodies**. In the liver, three prominent EGF-dependent substrates of M(r) .simeq. 170,000, 120,000, and 55,000 were detected. A number of less prominent EGF-dependent substrates also were noted. Maximal tyrosine **phosphorylation** of pp170, pp120, and pp55 occurred within 5 min of subcutaneous injection and the levels of these phosphoproteins remained elevated for at least 45 min. Direct hepatic injection of EGF resulted in the tyrosine **phosphorylation** of these substrates within 60 sec of treatment. Tyrosine-phosphorylated pp170 was identified as the EGF receptor (**EGFR**). The tyrosine-phosphorylated pp55 substrate appeared to be associated with **EGFR**; both pp55 and **EGFR** were adsorbed to EGF-Affi-Gel, wheat germ lectin-Sepharose, and anti-**EGFR antibodies** bound to protein A- Sepharose. pp55 was not immunoreactive with anti-**EGFR** antiserum by Western blot analysis, indicating that it was not a fragment of the receptor. These results were confirmed by repeating the liver experiments using 32P-labeled neonatal mice. Increased amounts of 32P-labeled pp170 and pp55 were detected in anti-**EGFR** immunoprecipitates from liver extracts of EGF-treated animals as compared with controls. Phospho amino acid analysis of the 32P- labeled phosphoproteins revealed that EGF stimulated both **serine** and tyrosine **phosphorylation** in pp55 as well as in **EGFR**. The neonatal mouse may be a useful model for the study of signal transduction mediated by a variety of growth factors.

```
=> s l5 and threonine
L8          15 L5 AND THREONINE

=> dup remove l8
PROCESSING COMPLETED FOR L8
L9             8 DUP REMOVE L8 (7 DUPLICATES REMOVED)

=> d l9 1-8 cbib abs
```

L9 ANSWER 1 OF 8 MEDLINE
2002285242 Document Number: 22021454. PubMed ID: 11992543. Effects of pharmacologic antagonists of **epidermal growth factor receptor**, PI3K and MEK signal kinases on NF-kappaB and AP-1 activation and IL-8 and VEGF expression in human head and neck squamous cell carcinoma lines. Bancroft Caren C; Chen Zhong; Yeh Jason; Sunwoo John B; Yeh Ning T; Jackson Sadhana; Jackson Chad; Van Waes Carter. (Tumor Biology Section, Head and Neck Surgery Branch, The National Institute on Deafness and Other Communication Disorders, The National Institutes of Health, Bethesda, MD 20892, USA.) INTERNATIONAL JOURNAL OF CANCER, (2002 Jun 1) 99 (4) 538-48. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.
AB We previously reported that expression of angiogenesis factors

interleukin-8 (IL-8) and vascular endothelial growth factor (VEGF) is promoted by coactivation of transcription factors nuclear factor-kappaB (NF-kappaB) and activator protein-1 (AP-1) by interleukin-1alpha in human head and neck squamous cell carcinomas (HNSCC). However, expression of IL-1 receptor antagonist incompletely blocked reporter gene activity and cytokine expression, suggesting that other upstream signals may contribute to activation. Overexpression and autocrine activation of **epidermal growth factor receptor** (**EGFR**) is detected in 90% of HNSCC, and **EGFR** inhibitors have been reported to inhibit IL-8 and VEGF expression, but the intermediary signal pathways and transcription factors by which **EGFR** modulates proangiogenic factors is unknown. **EGFR** can activate the phosphotidylinositol-3 kinase (PI3K) and mitogen-activated/extracellular signal-regulated kinase (MEK) pathways, which can potentially modulate activation of NF-kappaB and AP-1, respectively. In our study, we examined the effect of EGF and antagonists of **EGFR**, PI3K and MEK on NF-kappaB and AP-1 activation and IL-8 and VEGF expression in HNSCC cell lines UM-SCC-9 and 11B in which **EGFR** is overexpressed and activated. Recombinant EGF induced **EGFR phosphorylation**, activation of NF-kappaB and AP-1 reporter genes and IL-8 and VEGF expression, indicating that **EGFR** can mediate coactivation of both transcription factors and cytokine genes in HNSCC. **EGFR** antagonist PD153035 and anti-**EGFR** **antibody** C225 completely inhibited EGF-induced reporter activity and cytokine expression, but only partially inhibited constitutive activity. MEK inhibitor U0126 preferentially blocked AP-1 activity and expression of both IL-8 and VEGF, while PI3K inhibitor LY-294002 or a dominant negative inhibitor-kappaB preferentially blocked NF-kappaB activation and expression of IL-8 but not VEGF. **EGFR**, PI3K and MEK antagonists inhibited growth of HNSCC. We conclude that antagonists of **EGFR**, PI3K and MEK signal pathways have inhibitory activity against **EGFR**-induced NF-kappaB and AP-1 activation, IL-8 and VEGF expression and growth by HNSCC. Published 2002 Wiley-Liss, Inc.

L9 ANSWER 2 OF 8 MEDLINE

2002102880 Document Number: 21821829. PubMed ID: 11832328.

Vasopressin-mediated mitogenic signaling in intestinal epithelial cells. Chiu Terence; Wu Steven S; Santiskulvong Chintda; Tangkijvanich Pisit; Yee Hal F Jr; Rozengurt Enrique. (Department of Medicine, School of Medicine, University of California-Los Angeles, 900 Veteran Ave., Los Angeles, CA 90095, USA.) AMERICAN JOURNAL OF PHYSIOLOGY. CELL PHYSIOLOGY, (2002 Mar) 282 (3) C434-50. Journal code: 100901225. ISSN: 0363-6143. Pub. country: United States. Language: English.

AB The role of G protein-coupled receptors and their ligands in intestinal epithelial cell signaling and proliferation is poorly understood. Here, we demonstrate that arginine vasopressin (AVP) induces multiple intracellular signal transduction pathways in rat intestinal epithelial IEC-18 cells via a V(1A) receptor. Addition of AVP to these cells induces a rapid and transient increase in cytosolic Ca(2+) concentration and promotes protein kinase D (PKD) activation through a protein kinase C (PKC)-dependent pathway, as revealed by in vitro kinase assays and immunoblotting with an **antibody** that recognizes autophosphorylated PKD at Ser(916). AVP also stimulates the tyrosine **phosphorylation** of the nonreceptor tyrosine kinase proline-rich tyrosine kinase 2 (Pyk2) and promotes Src family kinase **phosphorylation** at Tyr(418), indicative of Src activation. AVP induces extracellular signal-related kinase (ERK)-1 (p44(mapk)) and ERK-2 (p42(mapk)) activation, a response prevented by treatment with mitogen-activated protein kinase kinase (MEK) inhibitors (PD-98059 and U-0126), specific PKC inhibitors (GF-I and Ro-31-8220), depletion of Ca(2+) (EGTA and thapsigargin), selective **epidermal growth factor receptor** (**EGFR**) tyrosine kinase inhibitors (tyrphostin AG-1478, compound 56), or the selective Src family kinase inhibitor PP-2. Furthermore, AVP acts as a

potent growth factor for IEC-18 cells, inducing DNA synthesis and cell proliferation through ERK-, Ca(2+)-, PKC-, **EGFR** tyrosine kinase-, and Src-dependent pathways.

L9 ANSWER 3 OF 8 MEDLINE

2000404731 Document Number: 20357377. PubMed ID: 10801894. Peroxynitrite targets the **epidermal growth factor receptor**, Raf-1, and MEK independently to activate MAPK. Zhang P; Wang Y Z; Kagan E; Bonner J C. (Laboratory of Pulmonary Pathobiology, NIEHS, National Institutes of Health, Research Triangle Park, North Carolina 27709, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Jul 21) 275 (29) 22479-86. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Activation of ERK-1 and -2 by H(2)O(2) in a variety of cell types requires **epidermal growth factor receptor** (**EGFR**) **phosphorylation**. In this study, we investigated the activation of ERK by ONOO(-) in cultured rat lung myofibroblasts. Western blot analysis using anti-phospho-ERK **antibodies** along with an ERK kinase assay using the phosphorylated heat- and acid-stable protein (PHAS-1) substrate demonstrated that ERK activation peaked within 15 min after ONOO(-) treatment and was maximally activated with 100 micrometer ONOO(-). Activation of ERK by ONOO(-) and H(2)O(2) was blocked by the antioxidant N-acetyl-l-cysteine. Catalase blocked ERK activation by H(2)O(2), but not by ONOO(-), demonstrating that the effect of ONOO(-) was not due to the generation of H(2)O(2). Both H(2)O(2) and ONOO(-) induced **phosphorylation** of **EGFR** in Western blot experiments using an anti-phospho-**EGFR antibody**. However, the **EGFR** tyrosine kinase inhibitor AG1478 abolished ERK activation by H(2)O(2), but not by ONOO(-). Both H(2)O(2) and ONOO(-) activated Raf-1. However, the Raf inhibitor forskolin blocked ERK activation by H(2)O(2), but not by ONOO(-). The MEK inhibitor PD98059 inhibited ERK activation by both H(2)O(2) and ONOO(-). Moreover, ONOO(-) or H(2)O(2) caused a cytotoxic response of myofibroblasts that was prevented by preincubation with PD98059. In a cell-free kinase assay, ONOO(-) (but not H(2)O(2)) induced autophosphorylation and nitration of a glutathione S-transferase-MEK-1 fusion protein. Collectively, these data indicate that ONOO(-) activates **EGFR** and Raf-1, but these signaling intermediates are not required for ONOO(-)-induced ERK activation. However, MEK-1 activation is required for ONOO(-)-induced ERK activation in myofibroblasts. In contrast, H(2)O(2)-induced ERK activation is dependent on **EGFR** activation, which then leads to downstream Raf-1 and MEK-1 activation.

L9 ANSWER 4 OF 8 MEDLINE

1999232131 Document Number: 99232131. PubMed ID: 10216485. Investigation of the Mek-MAP kinase-Rsk pathway in human breast cancer. Salh B; Marotta A; Matthewson C; Ahluwalia M; Flint J; Owen D; Pelech S. (Department of Medicine, University of British Columbia, Vancouver, Canada.) ANTICANCER RESEARCH, (1999 Jan-Feb) 19 (1B) 731-40. Journal code: 8102988. ISSN: 0250-7005. Pub. country: Greece. Language: English.

AB BACKGROUND: Mitogenic signaling through the principal growth factor receptor tyrosine kinase (RTK) pathway, i.e. RTK-->Ras-->Raf-->Mek-->MAPK has been implicated in the pathogenesis of human cancer. However, biochemical characterization of this has not been adequately assessed in human cancers. MATERIALS AND METHODS: Using extracts from 23 human breast cancers and control tissue from the same resected specimens, the protein levels, phosphotransferase activities and subcellular locations of the mitogen-activated protein (MAP) kinase isoforms p42 Erk2 and p44 Erk1 were examined, together with their phosphotransferase activities towards myelin basic protein (MBP) and a peptide substrate patterned after the Thr-669 site in the **epidermal growth factor receptor** (**EGFR** T669) that is phosphorylated by MAP kinase. RESULTS: Overexpression of both Erk2 and Erk1 isoforms was evident

using specific **antibodies**. A universal activation of MBP and **EGFR** T669 peptide phosphotransferase activities was also found (up to 3-fold). MonoQ fractionation resolved the bulk of the **EGFR** T669 peptide **phosphorylation** from elution of the MAP kinase protein. Erk1 and Erk2 activities determined by specific immunoprecipitation were increased by up to only 2.5-fold in only 50% of tumors overall. Immunohistochemical studies, using a monoclonal **antibody** specific for Erk2 demonstrated that the cellular distribution of this MAP kinase was similar in both control and tumor tissues, and Erk2 was largely confined to normal and malignant acini, whilst the intensity of staining was actually reduced in the tumor tissue. Mek1 and especially Mek2 protein expression, as well as MAP kinase kinase activity as determined by **phosphorylation** of kinase-inactive Erk [GST-K71A] were increased in cancer samples. CONCLUSIONS: a) This confirms that MAP kinase activity is increased in human breast cancer. However, the frequency and magnitude of this change is dependent upon the chosen methodology (i.e. crude lysate assays versus specific immunoprecipitation). b) A MAP-kinase-independent source of increased **EGFR** T669 phosphotransferase activity in tumor extracts has been demonstrated for the first time in human breast cancer. c) By immunohistochemistry, Erk2 protein was actually found to exhibit lower intensity in tumor samples; the increased expression was most likely due to its increased distribution. d) Increased Mek protein expression and activation have been demonstrated for the first time in human breast tumors.

L9 ANSWER 5 OF 8 SCISEARCH COPYRIGHT 2002 ISI (R)
96:673872 The Genuine Article (R) Number: VG672. MUTATIONAL ANALYSIS OF THE NUCLEOTIDE-BINDING SITE OF THE **EPIDERMAL GROWTH-FACTOR RECEPTOR** AND V-SRC PROTEIN-TYROSINE KINASES.
CHAN C L; GILL G N (Reprint). UNIV CALIF SAN DIEGO, DEPT MED, 9500 GILMAN DR, LA JOLLA, CA, 92093 (Reprint); UNIV CALIF SAN DIEGO, DEPT MED, LA JOLLA, CA, 92093. JOURNAL OF BIOLOGICAL CHEMISTRY (13 SEP 1996) Vol. 271, No. 37, pp. 22619-22623. ISSN: 0021-9258. Pub. country: USA. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Tyrosine kinases differ from serine/**threonine** kinases in sequences located at the active site where ATP and substrate bind. In the structure of cyclic AMP-dependent protein kinase, the catalytic loop contains the sequence Lys-Pro-Glu where the Lys residue contacts the gamma-phosphate of ATP and the Glu residue contacts a basic residue located in the peptide substrate. In tyrosine kinases, the analogous sequence is Ala-Ala-Arg in the receptor tyrosine kinase subfamily and Arg-Ala-Ala in the Src tyrosine kinase subfamily. To deduce the role of these residues in tyrosine kinase function, site-directed mutations were prepared in the **epidermal growth factor receptor** (**EGFR**) and in v-Src and effects on ATP binding and kinase activity were determined. Changing Arg to either Lys or Ala dramatically reduced activity of both tyrosine kinases and this correlated with loss of ATP binding. Changing the orientation of this sequence impaired activity of **EGFR** to a greater extent than that of v-Src but did not change substrate specificity of the two enzymes. These results support the hypothesis that Arg functions to coordinate the gamma-phosphate of ATP. Analysis of sequence inversions in the catalytic loop indicate that the active site of v-Src exhibits greater flexibility than that of **EGFR**.

L9 ANSWER 6 OF 8 MEDLINE DUPLICATE 1
96183364 Document Number: 96183364. PubMed ID: 8610433. The protein kinase activity of the large subunit of herpes simplex virus type 2 ribonucleotide reductase (ICP10) fused to the extracellular domain of the **epidermal growth factor receptor** is ligand-inducible. Smith C C; Luo J H; Aurelian L. (Department of

Pharmacology, University of Maryland School of Medicine, Baltimore 21201, USA.) VIROLOGY, (1996 Mar 15) 217 (2) 425-34. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The gene coding for the large subunit of herpes simplex virus type 2 ribonucleotide reductase (RR) (ICP10) has a unique 5' terminal domain the product of which has a serine/**threonine** (Ser/Thr) protein kinase (PK) catalytic domain preceded by a transmembrane (TM) segment. Because ICP10 localizes on the cell surface and is internalized by the endocytic pathway like an activated growth factor receptor (Hunter et al., 1995, Virology 210, 345-360), we asked whether it is ligand-inducible in order to examine whether it has intrinsic transphosphorylating activity. We constructed a chimeric expression vector that contains the extracellular and TM domains of the **epidermal growth factor receptor** (**EGFR**) joined to the intracellular PK and RR domains of ICP10 (pCH5) and established constitutively expressing cell lines in NIH3T3 2.2 cells that do not express **EGFR**. The chimeric protein, designated p210 CH5, localized to the surface of these cells as determined by immunofluorescent staining with MAb **EGFR**, and it bound 125I-EGF.p210 CH5 coprecipitated with protein species p170, p120, p88, p60, p44, p34, and p25. EGF treatment activated the PK activity of p210 CH5, resulting in its autophosphorylation and the **phosphorylation** of the p120, p88, and p34 species. Immunoprecipitation/immunoblotting with anti-ras-GAP **antibody** and phosphoamino acid analysis indicated that p120 is ras-GAP and it is phosphorylated on Ser/Thr residues. The identities of the phosphorylated p88 and p34 are still unknown. The data indicate that when fused to a ligand-regulated extracellular domain (**EGFR**), the ICP10 PK auto- and transphosphorylating activities are ligand-inducible. These findings support the interpretation that the ICP10 PK activity is intrinsic and indicate that ras-GAP is one of its **phosphorylation** substrates.

L9 ANSWER 7 OF 8 SCISEARCH COPYRIGHT 2002 ISI (R)

92:371621 The Genuine Article (R) Number: HY947. LIGAND-INDUCED ACTIVATION OF **EPIDERMAL GROWTH-FACTOR RECEPTOR** IN INTACT RAT MAMMARY ADENOCARCINOMA CELLS WITHOUT DETECTABLE RECEPTOR **PHOSPHORYLATION**. LICHTNER R B (Reprint); WIEDEMUTH M; KITTMANN A; ULLRICH A; SCHIRRMACHER V; KHAZAIE K. GERMAN CANC RES CTR, DEPT IMMUNOL & GENET, W-6900 HEIDELBERG 1, GERMANY; MAX PLANCK INST BIOCHEM, W-8033 MARTINSRIED, GERMANY. JOURNAL OF BIOLOGICAL CHEMISTRY (15 JUN 1992) Vol. 267, No. 17, pp. 11872-11880. ISSN: 0021-9258. Pub. country: GERMANY. Language: ENGLISH.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Expression and function of **epidermal growth factor receptor** (**EGFR**) was investigated in a metastatic cell clone (MTLn3) derived from the 13762NF rat mammary adenocarcinoma. No receptor **phosphorylation** could be identified in intact cells or in membrane preparations, while EGF-dependent **phosphorylation** of substrates occurred in intact cells. Indications for active suppression of receptor **phosphorylation** came from the fact that **EGFRs** bound in immunocomplexes or associated with the cytoskeleton of detergent treated cells were able to undergo basal and EGF-induced **phosphorylation** in vitro. Cross-linking experiments with I-125-EGF, as well as [S-35]methionine labeling followed by immunoprecipitation with receptor specific **antibodies** readily detected in MTLn3 cells the expected 170-kDa **EGFR** protein. In addition, two proteins with molecular masses of 420-480 and 95 kDa specifically bound I-125-EGF on intact MTLn3 and sparse cultures of A431 cells. **Phosphorylation** of the 420-480 kDa molecule could be identified in immunocomplexes of **EGFRs** isolated from MTLn3 and sparse A431 cells, but the 95-kDa receptor molecule was never phosphorylated. While the presence of alternative forms of **EGFR** in the highly metastatic cell clone MTLn3 was unexpected, our observations of inefficient receptor autophosphorylation

are in agreement with other recent reports and suggest that in MTLn3 cells **EGFR**-mediated signal transduction can be an event independent from receptor autophosphorylation.

L9 ANSWER 8 OF 8 MEDLINE DUPLICATE 2
90037242 Document Number: 90037242. PubMed ID: 2553748. Signal transduction by epidermal growth factor occurs through the subclass of high affinity receptors. Defize L H; Boonstra J; Meisenhelder J; Kruijer W; Tertoolen L G; Tilly B C; Hunter T; van Bergen en Henegouwen P M; Moolenaar W H; de Laat S W. (Hubrecht Laboratory, Netherlands Institute for Developmental Biology, Utrecht.) JOURNAL OF CELL BIOLOGY, (1989 Nov) 109 (5) 2495-507. Journal code: 0375356. ISSN: 0021-9525. Pub. country: United States. Language: English.

AB Many cell types display two classes of **epidermal growth factor receptor** (**EGFR**) as judged from EGF binding studies; i.e., a major class of low affinity **EGFR** and a minor class of high affinity **EGFR**. We have studied their respective contribution to the cascade of events elicited by EGF in human A431 carcinoma cells, using anti-**EGFR** mAb 2E9. This **antibody** specifically blocks EGF binding to low affinity **EGFR**, without activating receptors in intact cells, and thus enables us to study the effects of exclusive EGF binding to high affinity **EGFR**. We show that blocking of low affinity **EGFR** by mAb 2E9 has almost no effect on the activation of the receptor protein-tyrosine kinase by EGF, suggesting that **EGFR** kinase activation occurs exclusively through the subclass of high affinity **EGFR** (5-10%). In addition, we provide evidence that high affinity **EGFR** exists both in monomeric and dimeric forms, and that cross-**phosphorylation** of low affinity **EGFR** by high affinity **EGFR** may take place in dimers of both receptor types. We demonstrate that the following early cellular response to EGF are also unimpaired in the presence of mAb 2E9: (a) inositol phosphate production, (b) release of Ca2+ from intracellular stores, (c) rise in intracellular pH, (d) **phosphorylation** of EGF on **threonine** residue 654, (e) induction of c-fos gene expression, and (f) alteration in cell morphology. As possible nonspecific side effects, we observed that the EGF induced Ca2+ influx and fluid-phase pinocytosis were inhibited in A431 cells in the presence of mAb 2E9. We conclude, therefore, that the activation of the **EGFR** signal transduction cascade can occur completely through exclusive binding of EGF to the subclass of high affinity **EGFR**.

```
=> d his

     (FILE 'HOME' ENTERED AT 11:25:15 ON 05 AUG 2002)

     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 11:25:29 ON
     05 AUG 2002
L1      2332137 S ANTIBODY
L2         8371 S L1 AND EPIDERMAL GROWTH FACTOR RECEPTOR
L3         3081 S L2 AND EGFR
L4            0 S L3 AND THREONINE PHOSPHORYLATION
L5          553 S L3 AND PHOSPHORYLATION
L6           18 S L5 AND SERINE
L7           10 DUP REMOVE L6 (8 DUPLICATES REMOVED)
L8           15 S L5 AND THREONINE
L9            8 DUP REMOVE L8 (7 DUPLICATES REMOVED)

=> s l5 and VEGF production
L10           0 L5 AND VEGF PRODUCTION

=> s l5 and VEGF
```

L11 9 L5 AND VEGF

=> s l11 and inhibit
L12 5 L11 AND INHIBIT

=> dup remove l12
PROCESSING COMPLETED FOR L12
L13 1 DUP REMOVE L12 (4 DUPLICATES REMOVED)

=> d l13 cbib abs

L13 ANSWER 1 OF 1 MEDLINE DUPLICATE 1
2002285242 Document Number: 22021454. PubMed ID: 11992543. Effects of pharmacologic antagonists of **epidermal growth factor receptor**, PI3K and MEK signal kinases on NF-kappaB and AP-1 activation and IL-8 and **VEGF** expression in human head and neck squamous cell carcinoma lines. Bancroft Caren C; Chen Zhong; Yeh Jason; Sunwoo John B; Yeh Ning T; Jackson Sadhana; Jackson Chad; Van Waes Carter. (Tumor Biology Section, Head and Neck Surgery Branch, The National Institute on Deafness and Other Communication Disorders, The National Institutes of Health, Bethesda, MD 20892, USA.) INTERNATIONAL JOURNAL OF CANCER, (2002 Jun 1) 99 (4) 538-48. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB We previously reported that expression of angiogenesis factors interleukin-8 (IL-8) and vascular endothelial growth factor (**VEGF**) is promoted by coactivation of transcription factors nuclear factor-kappaB (NF-kappaB) and activator protein-1 (AP-1) by interleukin-1alpha in human head and neck squamous cell carcinomas (HNSCC). However, expression of IL-1 receptor antagonist incompletely blocked reporter gene activity and cytokine expression, suggesting that other upstream signals may contribute to activation. Overexpression and autocrine activation of **epidermal growth factor receptor** (**EGFR**) is detected in 90% of HNSCC, and **EGFR** inhibitors have been reported to **inhibit** IL-8 and **VEGF** expression, but the intermediary signal pathways and transcription factors by which **EGFR** modulates proangiogenic factors is unknown. **EGFR** can activate the phosphotidylinositol-3 kinase (PI3K) and mitogen-activated/extracellular signal-regulated kinase (MEK) pathways, which can potentially modulate activation of NF-kappaB and AP-1, respectively. In our study, we examined the effect of EGF and antagonists of **EGFR**, PI3K and MEK on NF-kappaB and AP-1 activation and IL-8 and **VEGF** expression in HNSCC cell lines UM-SCC-9 and 11B in which **EGFR** is overexpressed and activated. Recombinant EGF induced **EGFR phosphorylation**, activation of NF-kappaB and AP-1 reporter genes and IL-8 and **VEGF** expression, indicating that **EGFR** can mediate coactivation of both transcription factors and cytokine genes in HNSCC. **EGFR** antagonist PD153035 and anti-**EGFR antibody** C225 completely inhibited EGF-induced reporter activity and cytokine expression, but only partially inhibited constitutive activity. MEK inhibitor U0126 preferentially blocked AP-1 activity and expression of both IL-8 and **VEGF**, while PI3K inhibitor LY-294002 or a dominant negative inhibitor-kappaB preferentially blocked NF-kappaB activation and expression of IL-8 but not **VEGF**. **EGFR**, PI3K and MEK antagonists inhibited growth of HNSCC. We conclude that antagonists of **EGFR**, PI3K and MEK signal pathways have inhibitory activity against **EGFR**-induced NF-kappaB and AP-1 activation, IL-8 and **VEGF** expression and growth by HNSCC. Published 2002 Wiley-Liss, Inc.

=> s l5 and degradation

L14 32 L5 AND DEGRADATION

=> s l14 and EGF receptor
L15 21 L14 AND EGF RECEPTOR

=> dup remove l15
PROCESSING COMPLETED FOR L15
L16 11 DUP REMOVE L15 (10 DUPLICATES REMOVED)

=> d l16 1-11 cbib abs

L16 ANSWER 1 OF 11 MEDLINE DUPLICATE 1
2002175597 Document Number: 21853392. PubMed ID: 11865035. UV induces tyrosine kinase-independent internalisation and endosome arrest of the **EGF receptor**. Oksvold Morten P; Huitfeldt Henrik S; Ostvold Anne Carine; Skarpen Ellen. (Laboratory for Toxicopathology, Institute of Pathology, The National Hospital, University of Oslo, N-0027 Oslo, Norway.. m.p.oksvold@labmed.uio.no) . JOURNAL OF CELL SCIENCE, (2002 Feb 15) 115 (Pt 4) 793-803. Journal code: 0052457. ISSN: 0021-9533. Pub. country: England: United Kingdom. Language: English.
AB We have compared the activation and trafficking of **epidermal growth factor receptor** (**EGFR**) induced by UV light and EGF. Tyrosine **phosphorylation** of **EGFR** was not detected in UV-exposed cells by immunoblotting of whole cell lysates or **EGFR** immunoprecipitates with **antibodies** specific for each of the five activated autophosphorylation sites of **EGFR**. In addition, **EGFR** of UV-irradiated cells did not demonstrate increased (32)P-incorporation. However, UV-exposed cells demonstrated a gel mobility shift of **EGFR**, which was not abolished by alkaline phosphatase treatment. UV-exposure did not induce dimerisation of **EGFR**. Furthermore, UV induced internalisation of **EGFR** without polyubiquitination or **degradation**. UV-exposed **EGFR** was transferred to early endosomes and arrested in transferrin-accessible endosomes close to the cell surface. Whereas inhibition of the **EGFR** tyrosine kinase effectively inhibited tyrosine **phosphorylation** and internalisation of EGF-activated **EGFR**, internalisation of UV-exposed **EGFR** was unaffected. UV induced neither relocalisation of Shc and Grb2 nor activation of Raf, but activation of MEK and MAPK was observed. Our work indicates that UV induces internalisation of **EGFR** independent of its **phosphorylation** or receptor tyrosine kinase activation, and altered **EGFR** trafficking compared with ligand-activated receptor. In addition, MAPK activation by UV does not appear to be mediated by **EGFR** activation.

L16 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2002 ACS
2001:800049 Document No. 136:80254 Regulation of **epidermal growth factor receptor** signaling by endocytosis and intracellular trafficking. Burke, Patrick; Schooler, Kevin; Wiley, H. Steven (Department of Pathology, Division of Cell Biology and Immunology, University of Utah, Salt Lake City, UT, 84132, USA). Molecular Biology of the Cell, 12(6), 1897-1910 (English) 2001. CODEN: MBCEEV. ISSN: 1059-1524. Publisher: American Society for Cell Biology.
AB Ligand activation of the **epidermal growth factor receptor** (**EGFR**) leads to its rapid internalization and eventual delivery to lysosomes. This process is thought to be a mechanism to attenuate signaling, but signals could potentially be generated after endocytosis. To directly evaluate **EGFR** signaling during receptor trafficking, the authors developed a technique to rapidly and selectively isolate internalized **EGFR** and assocd. mols. with the use of reversibly biotinylated anti-**EGFR antibodies**. In addn., the authors developed

antibodies specific to tyrosine-phosphorylated **EGFR**.
With the use of a combination of fluorescence imaging and affinity pptn. approaches, the authors evaluated the state of **EGFR** activation and substrate assocn. during trafficking in epithelial cells. The authors found that after internalization, **EGFR** remained active in the early endosomes. However, receptors were inactivated before **degrdn.**, apparently due to ligand removal from endosomes. Adapter mols., such as Shc, were assocd. with **EGFR** both at the cell surface and within endosomes. Some mols., such as Grb2, were primarily found assocd. with surface **EGFR**, whereas others, such as Eps8, were found only with intracellular receptors. During the inactivation phase, c-Cbl became **EGFR** assocd., consistent with its postulated role in receptor attenuation. The authors conclude that the assocn. of the **EGFR** with different proteins is compartment specific. In addn., ligand loss is the proximal cause of **EGFR** inactivation. Thus, regulated trafficking could potentially influence the pattern as well as the duration of signal transduction.

L16 ANSWER 3 OF 11 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
2002130975 EMBASE Human VPS34 is required for internal vesicle formation within multivesicular endosomes. Futter C.E.; Collinson L.M.; Backer J.M.; Hopkins C.R.. C.R. Hopkins, Dept. of Biochemistry, Imp. Coll. of Science and Technology, London SW7 2AS, United Kingdom. colinhopkins@compuserve.ac.uk. Journal of Cell Biology 155/7 (1251-1263) 24 Dec 2001.
Refs: 60.
ISSN: 0021-9525. CODEN: JCLBA3. Pub. Country: United States. Language: English. Summary Language: English.
AB After internalization from the plasma membrane, activated **EGF receptors** (**EGFRs**) are delivered to multivesicular bodies (MVBs). Within MVBs, **EGFRs** are removed from the perimeter membrane to internal vesicles, thereby being sorted from transferrin receptors, which recycle back to the plasma membrane. The phosphatidylinositol (PI) 3'-kinase inhibitor, wortmannin, inhibits internal vesicle formation within MVBs and causes **EGFRs** to remain in clusters on the perimeter membrane. Microinjection of isotype-specific inhibitory **antibodies** demonstrates that the PI 3'-kinase required for internal vesicle formation is hVPS34. In the presence of wortmannin, **EGFRs** continue to be delivered to lysosomes, showing that their removal from the recycling pathway and their delivery to lysosomes does not depend on inward vesiculation. We showed previously that tyrosine kinase-negative **EGFRs** fail to accumulate on internal vesicles of MVBs but are recycled rather than delivered to lysosomes. Therefore, we conclude that selection of **EGFRs** for inclusion on internal vesicles requires tyrosine kinase but not PI 3'-kinase activity, whereas vesicle formation requires PI 3'-kinase activity. Finally, in wortmannin-treated cells there is increased EGF-stimulated tyrosine **phosphorylation** when **EGFRs** are retained on the perimeter membrane of MVBs. Therefore, we suggest that inward vesiculation is involved directly with attenuating signal transduction.

L16 ANSWER 4 OF 11 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:246433 Document No.: PREV200100246433. G protein-coupled receptors desensitize and down regulate **EGF receptor** in renal mesangial cells. Grewal, Jasjit S. (1); Raymond, John R. (1). (1) Strom Thurmond Bio-Med. Research Center, Medical Univ. Of South Carolina, 114 Daughty Street, BM 409, Charleston, SC, 29403 USA. FASEB Journal, (March 7, 2001) Vol. 15, No. 4, pp. A180. print. Meeting Info.: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001 Orlando, Florida, USA March 31-April 04, 2001
ISSN: 0892-6638. Language: English. Summary Language: English.
AB G protein coupled receptors (GPCR's) and receptor tyrosine kinases (RTK's)

signaling pathways, previously thought to be simple and exclusively compartmentalized, are recently emerging as extremely complex and involve intricate cross-talk. In this abstract we report for the first time that GPCR activation leads to desensitization and down regulation of the **epidermal growth factor receptor** (**EGFR**). 5-Hydroxytryptamine (5-HT), a ligand for 5-HT2A receptor induces concentration- and time-dependent activation of **EGFR** tyrosine **phosphorylation**. The PKC inhibitor, GF109203X blocked this transactivation while a specific MEK-1 inhibitor, PD 98059, didn't. Prolonged treatment (1h) with 1(micro)M 5-HT as well as other mitogenic GPCR ligands viz, bradykinin, lysophosphatidic acid, lead to a substantial decrease in the ability of multiple conc. of EGF (1-100ng/ml) to induce **phosphorylation** of **EGFR**. This diminished ability of EGF to induce **EGFR phosphorylation** after GPCR ligand treatment also leads to decreases in downstream signals like ERK **phosphorylation**, and activation of transcription factors like E2F1, CREB and NF-kB. 125I-EGF ligand binding assay as well as confocal laser microscopic studies using FITC-bound primary **antibody** raised against an external epitope of **EGFR** in mesangial cells, and rhodamine conjugated concanavalin A in **EGFR**-GFP transfected HEK cells demonstrated internalization and relocalization of **EGFR** on 5-HT treatment. This internalization of **EGF receptor** could be blocked by inhibitors of endocytosis (K+ depletion, hypertonic medium treatment, and chemical inhibitors like concanavalin A and monodansylcadaverine) as well as by co-transfection with mutant dynamin (K44A). Inhibition of protein synthesis supported a role for **degradation** of internalized receptors, as **EGF receptor** protein started decreasing after 20min of 5-HT treatment and disappeared completely after 150 min. In conclusion, multiple mitogenic GPCR's desensitize and downregulate **EGFR**'s through PKC and a process that appears to involve **EGFR** internalization.

L16 ANSWER 5 OF 11 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 2
2001:245025 The Genuine Article (R) Number: 409RK. Association of ErbB2 Ser(1113) **phosphorylation** with **epidermal growth factor receptor** co-expression and poor prognosis in human breast cancer. Ouyang X M; Gulliford T; Zhang H Y; Smith G; Huang G C; Epstein R J (Reprint). Natl Canc Ctr, Lab Tumor Phosphoproteom, 11 Hosp Dr, Singapore 169610, Singapore (Reprint); Natl Canc Ctr, Div Med Sci, Singapore 169610, Singapore; Univ London Imperial Coll Sci Technol & Med, Sch Med, Dept Metab Med, London, England; Univ London Imperial Coll Sci Technol & Med, Sch Med, Dept Oncol, London, England; Univ London Imperial Coll Sci Technol & Med, Sch Med, Dept Biochem, London, England; Univ London Imperial Coll Sci Technol & Med, Sch Med, Dept Urol, London, England. MOLECULAR AND CELLULAR BIOCHEMISTRY (FEB 2001) Vol. 218, No. 1-2, pp. 47-54. Publisher: KLUWER ACADEMIC PUBL. SPUIBOULEVARD 50, PO BOX 17, 3300 AA DORDRECHT, NETHERLANDS. ISSN: 0300-8177. Pub. country: Singapore; England. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The carboxyterminal domain of the **epidermal growth factor receptor** (**EGFR**) - a putative binding site for the ubiquitin ligase Cbl - is the site of serine **phosphorylation** events which are essential for ligand-dependent **EGFR** desensitization and **degradation**. Using a monoclonal **antibody** (aPS(1113)) which selectively recognizes the homologous phosphorylated domain in the ErbB2 oncoprotein, we show here that wild-type ErbB2 becomes Ser(1113)-phosphorylated following treatment of 3T3 cells with growth factors or tyrosine phosphatase inhibitors. In **EGFR**-overexpressing A431 cells, ligand-inducible aPS(1113) immunoreactivity declines more rapidly than other detectable **phosphorylation** events and is followed by **EGFR** downregulation. Analysis of 65 ErbB2-expressing primary breast cancers reveals a highly significant relationship between Ser(1113)

phosphorylation and **EGFR** overexpression ($p < 0.0001$) as well as an association with poor prognosis ($p = 0.005$). We submit that ErbB2 Ser(1113) **phosphorylation** status represents a novel and informative biomarker of cancer cell biology and tumor behavior.

L16 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2002 ACS
1999:163840 Document No. 130:321196 Transforming growth factor-alpha short-circuits downregulation of the **epidermal growth factor receptor**. Ouyang, Xiaomei; Gulliford, Timothy; Huang, Guocai; Esptein, Richard J. (Department of Metabolic Medicine, Imperial College School of Medicine, London, UK). Journal of Cellular Physiology, 179(1), 52-57 (English) 1999. CODEN: JCLLAX. ISSN: 0021-9541. Publisher: Wiley-Liss, Inc..

AB Transforming growth factor-alpha (TGF.alpha.) is an **epidermal growth factor receptor** (**EGFR**) ligand which is distinguished from EGF by its acid-labile structure and potent transforming function. The authors recently reported that TGF.alpha. induces less efficient **EGFR** heterodimerization and downregulation than does EGF. Here the authors use isoform-specific **EGFR** and ErbB2 **antibodies** to show that the duration of **EGFR** signaling induced by a single TGF.alpha. exposure is less than that induced by equimolar EGF. The protein trafficking inhibitor brefeldin A (BFA) reduces the duration of EGF signaling to an extent similar to that seen with TGF.alpha. alone; the effects of TGF.alpha. and BFA on **EGFR degrdn**. are opposite, however, with TGF.alpha. sparing **EGFR** from downregulation but BFA accelerating EGF-dependent receptor loss. This suggests that BFA blocks **EGFR** recycling and thus shortens EGF-dependent receptor signaling, whereas TGF.alpha. shortens receptor signaling and thus blocks **EGFR** downregulation. Consistent with this, repeated application of TGF.alpha. is accompanied by prolonged **EGFR** expression and signaling, whereas similar application of EGF causes receptor downregulation and signal termination. These findings indicate that constitutive secretion of pH-labile TGF.alpha. may perpetuate **EGFR** signaling by permitting early oligomer dissocn. and dephosphorylation within acidic endosomes, thereby extinguishing a phosphotyrosine-based downregulation signal and creating an irreversible autocrine growth loop.

L16 ANSWER 7 OF 11 SCISEARCH COPYRIGHT 2002 ISI (R)
1999:910350 The Genuine Article (R) Number: 257WY. In vitro endosome-lysosome transfer of dephosphorylated **EGF receptor** and Shc in rat liver. Authier F (Reprint); Chauvet G. UNIV PARIS 11, FAC PHARM, INSERM, U510, 5 RUE JEAN BAPTISTE CLEMENT, F-92296 CHATENAY MALABR, FRANCE (Reprint); HOP NECKER ENFANTS MALAD, INSERM, U30, F-75015 PARIS, FRANCE. FEBS LETTERS (12 NOV 1999) Vol. 461, No. 1-2, pp. 25-31. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 0014-5793. Pub. country: FRANCE. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We have studied the endosome-lysosome transfer of internalized **epidermal growth factor receptor** (**EGFR**) completes in a cell-free system from rat liver. Analytical subfractionation of a postmitochondrial supernatant fraction showed that a pulse of internalized [I-125]EGF was largely associated with a light endosomal fraction devoid of lysosomal markers. After an additional 30 min incubation in vitro in the presence of an ATP-regenerating system, the amount of [I-125]EGF in this compartment decreased by 39%, with an increase in I-125]EGF in lysosomes. No transfer of I-125]EGF to the cytosol was detected. To assess the fate of the internalized **EGFR** protein over the time coorse of the endo-lysosomal transfer of the ligand, the effect of a saturating dose of native EGF on subsequent lysosomal targeting of the **EGFR** was evaluated by immunoblotting, A massive translocation of the **EGFR** to the endosomal compartment was observed in response to ligand injection coincident with its tyrosine

phosphorylation and receptor recruitment of the tyrosine-phosphorylated adaptor protein Shc, During cell-free endosome-lysosome fusion, a time-dependent increase in the content of the **EGFR** and the two 55- and 46-kDa Shc isoforms was observed in lysosomal fractions with a time course super-imposable with with the lysosomal transfer of the ligand; no transfer of the 66-kDa Shc isoform was detected. The relationship between **EGFR** tyrosine kinase activity and **EGFR** sorting in endosomes investigated by immunoblot studies with anti-phosphotyrosine **antibodies** revealed that endosomal dephosphorylation of **EGFR** and Shc preceded lysosomal transfer. These results support the view that a lysosomal targeting machinery distinct from the endosomal receptor kinase activity, such as the recruitment of the signaling molecule Shc, may regulate this sorting event in the endosome. (C) 1999 Federation of European Biochemical Societies.

L16 ANSWER 8 OF 11 MEDLINE DUPLICATE 3
97172473 Document Number: 97172473. PubMed ID: 9020117. Subsets of **epidermal growth factor receptors** during activation and endocytosis. Emlet D R; Moscatello D K; Ludlow L B; Wong A J. (Department of Pharmacology, Kimmel Cancer Institute, Philadelphia, Pennsylvania 19107, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Feb 14) 272 (7) 4079-86. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Mutation of the autophosphorylation sites of receptor protein-tyrosine kinases alters ligand dependent internalization and down-regulation, indicating a critical role for these sites in receptor processing. Currently, no differences in receptor processing based on an individual autophosphorylation site have been defined. By using a glutathione S-transferase fusion protein containing the src homology 2 domains of phospholipase C-gammal to specifically recognize tyrosine 992 on the **EGF receptor** (Tyr(P)992), we have found differences in this subpopulation of receptors. Following EGF stimulation, the number of Tyr(P)992 receptors increased 2-fold over receptors identified by an **antibody** that recognizes activated **EGF receptors** (alpha-Act. **EGFR**) in A431 cells. Confocal fluorescence microscopy showed that Tyr(P)992 receptors underwent endocytosis at a slower rate and did not rapidly concentrate in juxtanuclear bodies. Tyr(P)992 receptors were associated with more SOS, Ras-GTPase activating protein, phosphatidylinositol 3-kinase, and SHPTP2/syp, but less Grb2, than receptors in the general population, and these receptors were more heavily phosphorylated than the general population of active receptors. These findings suggest that autophosphorylation status is relevant to the endocytosis, **degradation**, and effector molecule interaction of individual **EGF receptors**. Further investigations based on **phosphorylation** status should provide new insights into how receptor protein-tyrosine kinase signaling is regulated.

L16 ANSWER 9 OF 11 SCISEARCH COPYRIGHT 2002 ISI (R)
97:483900 The Genuine Article (R) Number: XF539. Protein kinase C inhibits **epidermal growth factor receptor** **phosphorylation** in enterocytes. Summers S T (Reprint); Bass B L. VET ADM MED CTR, DEPT SURG, BALTIMORE, MD 21201 (Reprint); UNIV MARYLAND, SCH MED, BALTIMORE, MD 21201. JOURNAL OF SURGICAL RESEARCH (APR 1997) Vol. 69, No. 1, pp. 208-211. Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS. 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495. ISSN: 0022-4804. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Epidermal growth factor (EGF) is an important proliferative signal in the gastrointestinal tract. The **EGF receptor** (**EGFr**), which transduces the mitogenic stimulus to the cell, may be regulated by a number of factors including extracellular matrix, cell-cell contact, and other peptides, As protein kinase C (PK-C) has been shown to

phosphorylate and down-regulate the **EGFr** in certain tumor cell lines, we propose that PK-C, an important regulatory enzyme, modulates the **phosphorylation** of the **EGFr** in the IEC 6 rat enterocyte cell line. IEC 6 cells were cultured in dishes with Dulbecco's modified Eagle's medium, (DMEM)/5% fetal bovine serum (FBS), which was changed to DMEM/1% FBS 24 in prior to all experiments, Cells (three dishes per group) were treated with the PK-C activating phorbol ester phorbol-12-myristate-13-acetate (PMA) (100 nM) or vehicle for 1 hr and challenged with EGF (50 ng/ml) or vehicle for 15 min. Cell lysates were then prepared **EGFr** tyrosine **phosphorylation** was determined by immunoprecipitating the **EGFr** and immunoblotting with an **antibody** against phosphotyrosine, **EGFr** apparent molecular weight was assessed in the same lysates by Western blot with an anti-**EGFr** **antibody**. Blots were analyzed by computer densitometry. Data are expressed as mean +/- SEM; n = 3 with P value determined by t test. Exposure of cells to PMA resulted in a decrease in the EGF-stimulated **EGFr** phosphotyrosine content from 96 +/- 5 U in control to 66 +/- 6 U in PMA ($P < 0.01$). The amount of receptor did not change, 43 +/- 3 U in control vs 44 t 3 U in PMA ($P = 0.44$). Further, exposure to PMA in the absence of EGF caused a gel shift of the **EGFr** band consistent with a nontyrosine **phosphorylation** of the protein. We demonstrate that activation of PKC results in a modification of the **EGFr** coincident with inhibition of EGF-stimulated receptor tyrosine kinase activity. These data support a role for PR-C in the regulation of EG;Fr function and hence modulation of mitogenic signals in enterocytes. (C) 1997 Academic Press.

L16 ANSWER 10 OF 11 MEDLINE
93323192 Document Number: 93323192. PubMed ID: 8392596. The E5 oncoprotein of human papillomavirus type 16 transforms fibroblasts and effects the downregulation of the **epidermal growth** **factor receptor** in keratinocytes. Straight S W; Hinkle P M; Jewers R J; McCance D J. (Department of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, Rochester 14642.) JOURNAL OF VIROLOGY, (1993 Aug) 67 (8) 4521-32. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB To determine the function of the E5 open reading frame (ORF) of the human papillomaviruses (HPVs), rodent fibroblast cell lines were transfected with the E5 ORF of HPV type 6 (HPV-6) and HPV-16 expressed from an exogenous promoter. Transfected fibroblasts were transformed to colony formation in soft agar, and the transformation frequency was increased by epidermal growth factor (EGF) but not by platelet-derived growth factor. In a transitory assay, the E5 ORFs from both HPV-6 and HPV-16 were mitogenic in primary human foreskin epithelial cells (keratinocytes) and acted synergistically with EGF. Investigation of keratinocytes expressing HPV-16 E5 showed that the number of endogenous **EGF** **receptors** (**EGFRs**) per cell was increased two- to fivefold. Immunofluorescence microscopy of HPV-16 E5-expressing keratinocytes indicated that there was an apparent delay in the internalization and **degradation** of **EGFRs** compared with controls. Kinetic studies with [125I]EGF showed that the ligand underwent normal internalization and **degradation** in both HPV-16 E5-expressing and control keratinocytes, but in E5-expressing cells, a greater number of receptors recycled back to the cell surface within 1 to 6 h of ligand binding. Finally, ligand-stimulated **phosphorylation** of the **EGFR** on tyrosine, an indication of receptor kinase activity, was of greater magnitude in the HPV-16 E5-expressing keratinocytes than in control cells, although the basal level of receptor **phosphorylation** was similar.

L16 ANSWER 11 OF 11 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 4
92:167053 The Genuine Article (R) Number: HH747. IDENTIFICATION AND BIOCHEMICAL-CHARACTERIZATION OF NOVEL PUTATIVE SUBSTRATES FOR THE

EPIDERMAL GROWTH-FACTOR RECEPTOR
KINASE. FAZIOLI F; BOTTARO D P; MINICHIELLO L; AURICCHIO A; WONG W T; SEGATTO O; DIFIORE P P (Reprint). NCI, CELLULAR & MOLEC BIOL LAB, BLDG 37, RM 1D23, BETHESDA, MD, 20892. JOURNAL OF BIOLOGICAL CHEMISTRY (15 MAR 1992) Vol. 267, No. 8, pp. 5155-5161. ISSN: 0021-9258. Pub. country: USA.
Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB To gain insight into the mechanisms which control the mitogenic response to epidermal growth factor (EGF), we have partially purified and characterized several intracellular proteins which are phosphorylated on tyrosine residues following activation of the **epidermal growth factor receptor** (**EGFR**).
Partial purification was achieved by immunoaffinity chromatography using immobilized anti-phosphotyrosine **antibodies**. Antisera generated against the partially purified proteins were used to identify at least five novel **EGFR** putative substrates, designated, on the basis of their apparent molecular weight, p97, p68, p61, p56, and p23. All of these proteins became specifically phosphorylated on tyrosine after EGF treatment of intact cells, as assessed by phosphoamino acid analysis, and none of them represented an **EGFR degradation** product.
The **phosphorylation** of these proteins appeared to be relatively specific for the **EGFR**. In particular, an **EGFR**-related kinase, erbB-2 was much less efficient than **EGFR** at phosphorylating p97, p56, and p23 and incapable of phosphorylating p68.
The identification of these novel **EGFR** putative substrates should lead to a better understanding of the mechanisms controlling the specificity of **EGFR**-mediated mitogenic signaling.

```
=> s l5 adn 63 kD
MISSING OPERATOR L5 ADN
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l5 and 63 kD
L17          0 L5 AND 63 KD

=> s (jakobovits a? or yang x? or yang d?/au or gallo m?/au)
TERM 'A?' EXCEEDED TRUNCATION LIMITS - SEARCH ENDED
   1 FILES SEARCHED...


---Logging off of STN---


END
u
SEARCH ENDED BY USER
   2 FILES SEARCHED...
SEARCH ENDED BY USER
You have entered a truncated stem which occurs in too many terms.
Make the stem longer and try again.  For example, if your original
term was 'degr?' to search for variations and the abbreviation for
'degradation', you could replace it with the expression '(degrdn OR
degrad?)'.  If your search term was numeric, e.g., 'C>5', reduce the
size of the range.

=>
Executing the logoff script...


=> LOG Y
```

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	93.69	93.90

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-1.24	-1.24

STN INTERNATIONAL LOGOFF AT 11:33:18 ON 05 AUG 2002

Access DB# 36829

SEARCH REQUEST FORM

Scientific and Technical Information Center

Requester's Full Name: _____ Examiner #: _____ Date: _____

Art Unit: _____ Phone Number 30 _____ Serial Number: _____

Mail Box and Bldg/Room Location: _____ Results Format Preferred (circle): PAPER DISK E-MAIL

If more than one search is submitted, please prioritize searches in order of need.

****

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: _____

Inventors (please provide full names): _____

_____

Earliest Priority Filing Date: _____

For Sequence Searches Only *Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.*

****

STAFF USE ONLY	Type of Search	Vendors and cost where applicable
Searcher:	NA Sequence (#)	STN:
Searcher Phone #:	AA Sequence (#) 3	Dialog:
Searcher Location:	Structure (#)	Questel/Orbit:
Date Searcher Picked Up: 3/12	Bibliographic	Dr.Link:
Date Completed: 5/12	Litigation	Lexis/Nexis:
Searcher Prep & Review Time:	Fulltext	Sequence Systems:
Clerical Prep Time: 15	Patent Family	WWW/Internet:
Online Time: 20	Other	Other (specify):

PTO-1590 (1-2000)